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Structural Studies of Prokaryotic Transcription

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Beamline(s): X25, X9A

Introduction: Transcription is the major control point of gene expression and RNA polymerase (RNAP) is the central enzyme of transcription. Our long term goal is to understand the mechanism of transcription and its regulation. Determining three-dimensional structures of RNAP and its complexes with DNA, RNA, and regulatory factors is an essential step. This is best accomplished with highly characterized prokaryotic RNAPs, especially because of the high degree of conservation of RNAP structure and function from bacteria to man.

To this end, we determined the 3.3 Å-resolution crystal structure of a prokaryotic RNAP, the 380 kDa core RNAP from the thermophilic eubacteria *Thermus aquaticus* (Taq; subunit composition $\alpha_2\beta\beta'\omega$) and employed extensive crosslinking experiments to construct a model of the ternary elongation complex containing core RNAP, DNA template, and RNA transcript (1-3). We also solved the co-crystal structure of RNAP with rifampicin, an important antibiotic inhibitor (2). Our current work is aimed towards adding to our understanding of the enzyme's function and its regulation.

Results: We have two main lines of research, one on the RNAP itself (conducted mainly at X25), and on the promoter specificity σ factors (conducted mainly at X9A):

Structural studies of RNAP

1. We solved the crystal structure of the initiating form of *Taq* RNAP, containing core RNAP and the promoter specificity σ subunit, at 4 Å-resolution (4). Important structural features of the RNAP and their roles in positioning σ within the initiation complex are delineated. The two C-terminal domains of σ are separated by 45 Å on the surface of the RNAP, but are linked by an extended loop. The loop winds near the RNAP active site, where it may play a role in initiating nucleotide substrate binding, and out through the RNA exit channel. The advancing RNA transcript must displace the loop, leading to abortive initiation and ultimately to σ release.

2. We solved the crystal structure of *Taq* RNAP holoenzyme complexed with a promoter DNA fragment by fitting high-resolution X-ray structures of individual components into a 6.5 Å resolution map (5). The DNA lies across one face of the holoenzyme, completely outside the RNAP active site channel. All sequence-specific core promoter contacts are mediated by σ. A universally conserved tryptophan is ideally positioned to stack on the exposed face of the base pair at the upstream edge of the transcription bubble. Universally conserved basic residues of σ provide critical contacts with the DNA phosphate backbone and play a role in directing the melted DNA template strand into the RNAP active site. The structure explains how holoenzyme recognizes promoters containing variably spaced –10 and –35 elements, and provides the basis for models of the closed and open promoter complexes.

Stuctural studies of RNAP σ factors

1. Proteolysis of Taq σ^A, which occurred in situ during crystallization, reveals three domains, σ_2, σ_3, and σ_4, connected by flexible linkers. Crystal structures of each domain were determined, as well as of σ_4 complexed with –35 element DNA (6). Exposed surfaces of each domain are important for RNAP binding. Universally conserved residues important for –10 element recognition and melting lie on one face of σ_2, while residues important for extended –10 recognition lie on σ_3. Genetic studies correctly predicted that a helix-turn-helix motif in σ_4 recognizes the –35 element, but not the details of the protein-DNA interactions. Positive control mutants in σ_4 cluster in two regions, positioned to interact with activators bound just upstream or downstream of the –35 element.

2. Cell type-specific transcription during Bacillus sporulation is established by σ^F. Negative regulation of σ^F is orchestrated by SpoIIAB, an anti-σ that binds σ^F, as well as a serine kinase that phosphorylates and inactivates the anti-anti-σ SpoIIAA. The crystal structure of σ^F bound to the SpoIIAB dimer in the low-affinity, ADP form has been determined at 2.9 Å resolution. SpoIIAB adopts the GHKL superfamily fold of ATPases and histidine kinases, with an N-terminal extension that forms the dimer interface. A domain of σ^F contacts both SpoIIAB monomers, while 80% of the σ factor is disordered. The interaction occludes an RNAP binding surface of σ^F, explaining the SpoIIAB anti-σ activity. The structure also explains the specificity of SpoIIAB for its target σ factors and, in combination with genetic and biochemical data, provides insight into the mechanism of SpoIIAA anti-anti-σ activity.

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